REMARKS

Reconsideration and allowance are respectfully requested.

Claims 1-22 are pending. Non-elected claims 9-14, 19 and 21-22 were withdrawn from consideration by the Examiner.

The amendments are supported by the original disclosure and, thus, no new matter has been added. For example, claims 12-14 are amended in accordance with page 21, line 28, to page 22, line 6, of the specification. But if the Examiner should disagree, she is respectfully requested to point out the challenged limitation with particularity in the next Action so support may be cited in response.

The Examiner has required election of a single primer pair to which claims 16 and 18 will be restricted. With respect to the election of species, Applicants submit that this election is scientifically improper for the following reasons. Localization of mutations in the nCL1 gene may be used to detect LGMD2 disease. A mutation is a modification within a gene, this modification and its position not being known a priori. Therefore the entire sequence of the gene may need to be scanned to localize the mutation. Thus the method of detecting LGMD2 disease may require the amplification of each of 103 nucleic acid segments (Tables 1-3) representing the entire nCL1 gene. Consequently, the amplification of any one nucleic acid segment (i.e., the use of one primer pair) may not be sufficient to detect a mutation in the nCL1 gene if the mutation is outside the amplified segment. In fact electing one pair of primers for amplifying only one segment of the nCL1 gene, either in claim 16 or 18, will render the method of claim 15 difficult to practice because the probability is very low that any one of these particular amplified segments contains the mutations leading to LGMD2 disease. To set the record straight, the above commentary concerning the election of species in no way or manner reflects a traversal of this requirement and should in no way be interpreted to argue that non-elected species are not patentable, if this election is not maintained. Rather the above solely offers a scientific explanation as to why Applicants deem the election scientifically improper and to request that the Examiner reconsider the election of a single pair of nucleotide sequences. But if the Examiner maintains this requirement, Applicants elect primers 62 and 63.

The Examiner has maintained that claims 5-7 and 20 c) do not share the same technical feature as claims 1-4, 8 and 20 a). Under PCT Rule 13.2, the requirement for unity of invention is satisfied when there is a technical relationship among the claimed inventions (i.e., the same technical feature). Claims 5-7 and 20 c) are drawn to amino acid sequences which are deduced directly and without any ambiguity from nucleotide sequences recited in claims 1-4 and 20 a). Thus, even if they are structurally distinct, they are functionally related and dependent. Reconsideration is requested.

Upon allowance of the product claims, Applicants request rejoinder of the method claims depending therefrom. Claims 12-14 and 22 have been amended.

An Abstract of the Disclosure is submitted herewith in response to the objection to the specification. No new matter is added because it is identical to the abstract of Intl. Patent Appln. No. PCT/EP95/04575. Withdrawal of the objection is requested.

Copies of the Table of Contents for the text cited as "<<ADN médicament>>, (John Libbey, Eurotext, 1993)" and its English translation are attached in response to the Examiner's request. This book is cited in the specification by Applicants for its description of gene therapy and was mentioned in the context of its description of vector systems including adenoviruses and retroviruses. Thus the Examiner will appreciate that the reference is not relevant to the claimed nucleotide sequences.

A substitute Sequence Listing is submitted herewith containing the sequences in Table 2 in response to the Examiner's objection. The paper and computer readable forms of the Sequence Listing do not add new matter, and their contents are the same. The brief description of Figure 2 has been corrected, the sequence identifiers and the accession number have been added, and citations have been completed. Copies of the Richard et al. (1995) and the Allamand et al. (1995) references are enclosed for the Examiner's consideration. Withdrawal of the objections to the disclosure is requested.

Amended claim 22 is a proper multiple dependent claim. Withdrawal of the objection to the claim is requested.

Claims 1-4, 8 and 20 were rejected under Section 101 because they are allegedly directed to products of nature. Applicants traverse because the claims have been

amended in accordance with the Examiner's suggestion of adding "isolated" before the nucleic acid sequence. Withdrawal of the Section 101 rejection is requested.

Claims 1-4, 8 and 20 were rejected under Section 112, second paragraph, as being allegedly "indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." Applicants traverse.

The parts of claim 1 have been designated by lower case letters and part 3) has been deleted as suggested by the Examiner.

The term "derived" has been replaced with --obtained-- as suggested by the Examiner.

Amended claim 3 is directed to a vector as suggested by the Examiner.

Claims 8 and 15 have been amended as suggested by the Examiner.

Claim 20 has not been amended because it is submitted that the nucleic acid and amino acid sequences share a special technical feature. Reconsideration is requested.

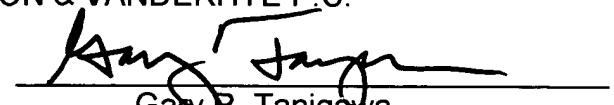
The Examiner's suggestions for amending the claims to correct informalities are gratefully acknowledged. Adoption of her suggestions moots certain rejections and their withdrawal is requested.

Conclusion

Having fully responded to all of the pending objections and rejections contained in the Office Action (Paper No. 32), Applicants submit that the claims are in condition for allowance and earnestly solicit an early Notice to that effect. The Examiner is invited to contact the undersigned if any further information is required.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: 

Gary R. Tanigawa
Reg. No. 43,180

1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

APPENDIX
MARKED-UP VERSION TO SHOW CHANGES

IN THE SPECIFICATION

The specification is amended as follows.

Page 5, replace the heading on line 15 with:

[Legend of the figures:] Brief Descriptions of the Drawings

Page 5, replace the fourth paragraph starting on line 15 with:

Figures 1A-1C:

A) Genomic organisation of the nCL1 gene

The gene covers a 40 kb region of which 35 were sequenced (Accession number [pending] NT 030828 Homo sapiens chromosome 15). Introns and exons are drawn to scale, the latter being indicated by numbered vertical bars. The first intron is the largest one and remains to be fully sequenced. Position of intragenic microsatellites are indicated by asterisks. Arrows indicate the orientation of Alu (closed) and of Mer2 (greyed) repeat sequences.

Page 5, replace the sixth paragraph starting on line 29 with:

Cosmids were from a cosmid library constructed by subcloning YAC 774G4 (Richard et al., 1995 [in preparation]) and are presented as lines. Dots on lines indicate positive STSs (indicated in boxed rectangles). A minimum of three cosmids cover the entire gene.

Page 6, replace the first paragraph starting on line 1 with:

Figures 2A-2C: Sequence of the human nCL1 cDNA (B), and the flanking 5' (A) and 3' (C) genomic regions.

A) (SEQ ID NO:68) and C) (SEQ ID NO:69) The polyadenylation signal and putative CAAT, TATAA sites are boxed. Putative Sp1 (position -477 to -472), MEF2 binding sites

(-364 to -343) and CArG box (-685 to -672) are in bold. The Alu sequence present in the 5' region is underlined.

B) The corresponding amino acids are shown below the sequence. The coding sequence between the ATG initiation codon and the TGA stop codon is 2466 bp ([positions 1303-3746 of]SEQ ID NO:70[5]), encoding for an 821 amino acid protein (SEQ ID NO:6). The adenine in the first methionine codon has been assigned position 1. Locations of introns within the nCL1 gene are indicated by arrowheads. Nucleotides which differ from the previously published ones are indicated by asterisks.

Page 6, replace the third paragraph starting on line 24 with:

Figures 4A-4B: Distribution of the mutations along nCL1 protein structure.

Page 7, replace the third paragraph starting on line 9 with:

Figures 7A-7D: Homozygous mutations in the nCL1 gene

Page 7, replace the fourth paragraph starting on line 15 with:

Figures 8A-8D: Structure of the nCL1 gene

Page 11, replace the second paragraph starting on line 3 with:

Table 2: Sequences at the intron-exon junctions (SEQ ID NO:71-SEQ ID NO:116). A score expressing adherence to the consensus was calculated for each site according to Shapiro and Senapathy (1987). Sequences of exons and introns are in upper and lower cases, respectively. Size of exons are given in parentheses [parenthesis].

Page 16, replace the sixth paragraph starting on line 30 with:

As expected, due to multiple consanguineous links, the examined LGMD2A Northern Indiana Amish patients were homozygous for the haplotype on the chromosome bearing the mutant allele (Allamand[,] et al., [submitted]1995). A (G->A) missense mutation was identified at nucleotide 2306 within exon 22 (Fig. 7). The resulting codon change is CGG to GAG, transforming Arg^{769} to glutamine. This residue,

which is conserved throughout all members of the calpain family in all species, is located in domain IV of the protein within the 3rd EF-hand at the helix-loop junction [(ref)]. This mutation was encountered in a homozygous state in all patients from 12 chromosome 15-linked Amish families, in agreement with the haplotype analysis. We also screened six Southern Indiana Amish LGMD families, for which the chromosome 15 locus was excluded by linkage analyses (Allamand ESHG, submitted, ASHG 94). As expected, this nucleotide change was not present in any of the patients from these families, thus confirming the genetic heterogeneity of this disease in this genetically related isolate.

Page 24, insert before the first paragraph starting on line 2:

Allamand, V., Broux, O., Richard, I., Fougerousse, F., Chiannilkuchai, Bourg, N., Brenguier, L., Devaud, C., Pasturaud, P., Pereira de Souza, A., Roudaut, C., Tiscfield, J. A., Connealy, P. M., Fardeau, M., Cohen, D., Jackson, C. E. and Beckmann, J. S. (1995). Preferential localization of the limb girdle muscular distrophy type 2A gene in the proximal part of a 1-cM 15q15.1-q15.3 interval. Am. J. Hum. Genet. *56*,1417-1430.

Page 28, insert after the first paragraph starting on line 3:

Richard, I., Roudaut, C., Fougerousse, F., Chiannilkuchai, N. and Beckmann, J. S. (1995). An STS map of the limb girdle muscular dystrophy type 2A region. Mammalian Genome *6*, 754-756.

IN THE CLAIMS

The claims are amended as follows.

1. (2x Amended) An isolated nucleic acid sequence comprising:
   [1)] (a) the sequence represented in Figure 8 (SEQ ID NO:1-SEQ ID NO:4); or
   [2)] (b) the sequence represented in Figure 2 (SEQ ID NO:5, SEQ ID NO:68 and SEQ ID NO:69); or

[3) a part of the sequence of Figure 2 (SEQ ID NO:5, SEQ ID NO:68 and SEQ ID NO:69) with the proviso that it is able to code for a protein having a calcium dependant protease activity involved in a LGMD2 disease; or]

[4)] (c) a sequence [derived] obtained from a sequence defined in a[1), 2]) or b[3]) by substitution, deletion or addition of one or more nucleotides with the proviso that said sequence still codes for said protease.

2. (Amended) An isolated nucleic acid sequence that is complementary to a nucleic acid sequence according to claim 1.

3. (Amended) A recombinant vector [nucleic acid sequence] comprising in its structure a [nucleotidic] nucleotide sequence according to claim 1, under the control of regulatory elements, and involved in the expression of calpain activity in a LGMD2 disease.

4. (2x Amended) An isolated nucleic acid sequence encoding the amino acid sequence represented in Figure 2 (SEQ ID NO:6).

5. (Amended) An isolated amino acid sequence which is encoded by a nucleic acid sequence according to Claim 1, characterized in that it is a calcium dependent protease enzyme belonging to the calpain family, involved in the etiology of LGMD2.

6. (3x Amended) An isolated amino acid sequence according to claim 5 characterized in that either it contains the sequence such as represented in Figure 2 (SEQ ID NO:6), or the amino acid sequence of Figure 2 (SEQ ID NO:6) modified by deletion, insertion and/or replacement of one or more amino acids with the proviso that such amino acid sequence has the calpain activity involved in LGMD2 disease.

7. (Amended) An isolated amino acid sequence according to claim 5, characterized in that LGMD2 is LGMD2A.

8. (Amended) A host cell unable to express a calpain enzyme activity, characterized in that it is transformed or transfected with a nucleic acid sequence comprising [all or part of] the isolated nucleic acid sequence according to Claim 1.

12. (Amended) A method of screening, such method comprising the steps of: [Use of]

- providing an isolated amino acid sequence according to Claim 5 and [for the screening of the]

- determining ligands of said amino acid sequence, said ligands being selected from the [in a] group consisting of substrate(s), co-factors and [or] regulatory components.

13. (Amended) A method of screening, such method comprising the steps of: [Use of]

- providing an isolated nucleic acid sequence according to Claim 1 and [in a screening method for the determination of the]

- determining components which regulate [may act on the regulation of gene] expression of the novel calpain large subunit 1 (nCL1) gene.

14. (Amended) A method of screening, such method comprising the steps of: [Use of]

- providing an host cell according to claim 8 and [in a screening method for the determination of]

- determining components which regulate [active on the] expression of the novel calpain large subunit 1 (nCL1) gene.

15. (2x Amended) A method for detecting [of a predisposition to a] an LGMD2 disease [in a family or a human being], such method comprising the steps of:

- selecting nucleotide sequences from one or more exons or [their] flanking sequences of [the] said one or more exons from an nCL1 gene,

- selecting primers specific for [these] said one or more exons, or [their] said flanking sequences, [or] of said one or more exons [an hybrid thereof],

- amplifying [the] nucleic acid sequences of said one or more exons or said flanking sequences of one or more exons with said selected primers, [with these primers, the substrate for this amplification being the DNA of a human being;] and

- comparing the amplified sequence to the corresponding sequence [derived] obtained from Figure 2 (SEQ ID NO:5, SEQ ID NO:68 and SEQ ID NO:69) or Figure 8 (SEQ ID NO:1-SEQ ID NO:4) wherein a mutation in said amplified sequences is indicative of an LGMD2 disease.

16. (2x Amended) The method according to claim 15, characterized in that the primers are those selected from the group consisting of:

a) those described in Table 1 (SEQ ID NO:10-SEQ ID NO:17);
b) those described in Table 3 (SEQ ID NO:18-SEQ ID NO:67); [and]
c) those including the introns-exons junctions of Table 2 (SEQ ID NO:71-SEQ ID NO:116); and
d) those derived from the primers in a), b), or c).

18. (2x Amended) A kit for the detection of a predisposition to LGMD2 by nucleic acid amplification characterized in that it comprises primers selected from the group consisting of:

a) those described in Table 1 (SEQ ID NO:10-SEQ ID NO:17);
b) those described in Table 3 (SEQ ID NO:18-SEQ ID NO:67); [and]
c) those including the introns-exons junctions of Table 2 (SEQ ID NO:71-SEQ ID NO:116); and
d) those derived from the primers in a), b), or c).

20. (2x Amended) A p[P]harmaceutical composition for the treatment of an LGMD2 disease characterized in that it contains a component selected from the group consisting of:

a) an isolated nucleic acid sequence according to claim 1,
b) a host cell according to claim 8, and

c) an isolated amino acid sequence according to claim 5.

22. (Amended) A method of screening a compound for its ability to modify the expression of the novel calpain large subunit 1 (nCL1) gene [nucleic acid sequence according to claim 1] comprising contacting [of] said compound with a host cell according to claim 8 and determining whether said compound modifies [the] expression of said nCL1 gene [nucleic acid sequence] in said host cell.

IN THE ABSTRACT

The Abstract of the Disclosure is attached.

IN THE SEQUENCE LISTING

Paper and computer readable copies of the Sequence Listing are attached.